PATENT ABSTRACTS OF JAPAN

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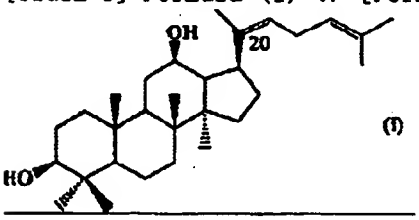
CLAIMS

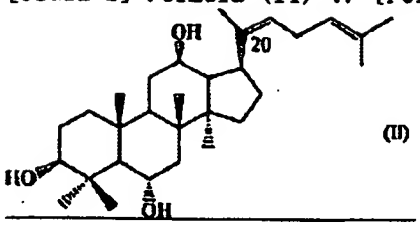
[Claim(s)]
[Claim 1] Formula (I) :. [Formula 1]



(I)

DAMARA -20 (22), 24-diene which are come out of and expressed - 3beta and 12beta-diol.

[Claim 2] Formula (II) :. [Formula 2]



(II)

DAMARA -20 come out of and expressed, (22), 24-diene - 3beta, 6alpha, 12beta-triol.

[Claim 3] DAMARA -20 (22), 24-diene which are expressed with a formula (I) according to claim 1 - DAMARA -20 expressed with a 3beta and 12beta-diol or a formula (II) according to claim 2, (22), 24-diene - Reinforcement agent for increasing the susceptibility of the cancer over the anticancer agent which makes an active principle 3beta, 6alpha, and 12beta-triol.

[Claim 4] The glycoside which makes a frame 20(S)-protopanaxadiol and 20(S)-protopanaxadiol DAMARA -20 expressed with the formula (I) according to claim 1 obtained as a sapogenin by hydrolyzing and dehydration processing (22), 24-diene - DAMARA -20 expressed with a 3beta and 12beta-diol and a formula (II) according to claim 2, (22), 24-diene - Manufacturing method of 3beta, 6alpha, and 12beta-triol.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Industrial Application] this invention relates to the reinforcement agent for increasing the susceptibility of the cancer over the anticancer agent which makes an active principle a new ginseng sapogenin, its manufacturing method, and its new ginseng sapogenin.

[0002]

[Description of the Prior Art] Since the susceptibility of Kanji by use of the chemotherapic drug of leukemia, a lymphoma, and various cancers like a solid cancer over each anticancer agent of a cancer cell is uneven, he is rare. The chemotherapy of cancer goes wrong also for the endogenicity resistance to the ** agent treatment of cancer. In other

cases, there are resistance and a bird clapper to the anticancer agent for which cancer was used in former treatment. The curative effect of these medicines is then removed. A still more important problem may show resistance also to other anticancer agents which are unrelated to the medicine with which the cancer which recurred was before used by either about [that it is resistance] the chemical structure or the action mechanism to the anticancer agent used in former treatment. These phenomena are put together, are called drug resistance, and are participating in clinical cancer treatment widely.

[0003]

[Problem(s) to be Solved] In the present condition of such cancer chemotherapy, in order to reduce side effects, such as bone marrow suppression which conquers drug resistance and an anticancer agent has, it is pressing need to develop the medicine which reinforces the effectiveness of an anticancer agent. They are verapamil [Tsuruo and T. as a thing typical to the medicine used for this purpose.; Cancer Res. 41, 1967 (1981)], and cyclosporin A[Slater, L.; Although there are J. Clin. Invest. 77 and 1405 (1986)], clinical application is made difficult for the side effect. They separated the rough saponin of a medicinal ginseng from the decomposition product obtained hydrolysis and by carrying out dehydration, the artificers of this invention were process search the medicine which reinforces the effect of a medicine of an anticancer agent, when the new ginseng sapogenin of structure non-Ming which named it a mulberry JIPANAKISA diol and mulberry JIPANAKISA triol increases the susceptibility of the cancer over an anticancer agent, they canceled drug resistance, and they found out reinforcing the chemotherapy-effect of an anticancer agent remarkably. Then, the chemical structure of the ginseng sapogenin of the structure non-Ming was determined, and since the method that they could be produced so much industrially was invented, it came to complete this invention with the operation which reinforces the effect of a medicine of those anticancer agents.

[0004]

[Means for Solving the Problem] That is, this invention is formula (I): which is a new ginseng sapogenin. [Formula 1] DAMARA -20 (22), 24-diene which came out and were named the mulberry JIPANAKISA diol (quasipanaxadiol) expressed - Offering a 3beta and 12beta-diol (dammara-20 (22) 24-diene-3beta, 12 beta-diol), this sapogenin has the following features.

1) The melting point is 150-153 degrees C.

2) [alpha] D20 It has the optical activity of +15 degree (C= 1.0,

methanol).
3) It has molecule composition of C30H5002.
4) An infrared absorption spectrum (KBr, cm-1) has 3400, 2900, 1450, 1380, and the absorption maximum peculiar to 1020.
5) A mass analysis spectrum (Fab, m/z) shows 441- (M-H).
7) 13C nuclear-magnetic-resonance spectrum (d5-pyridine) 39.3 (C-1), 27.0 (C-2), 78.5 (C-3), 40.4 (C-4), 56.4 (C-5), and 18.5 (C-6), 35.3 (C-7), 37.1 (C-8), and 50.9 (C-9), 39.6 (C-10), 32.2 (C-11), and 71.9 (C-12), 50.4 (C-13), 51.2 (C-14), and 32.6 (C-15), 26.8 (C-16), 51.2 (C-17), and 16.8 (C-18), 16.5 (C-19), 140.2 (C-20), and 27.4 (C-21), The signal of 123.8 (C-22), 30.0 (C-23), 125.4 (C-24), 131.5 (C-25), 25.7 (C-26), 17.7 (C-27), 28.2 (C-28), 15.8 (C-29), and 17.0 (C-30) is shown.
8) There is no smell and it is colorless needle crystal (from methanol-water to crystallization).
9) It is [a methanol, ethanol, n-butanol, 2-propanol, n-propanol a pyridine, dimethyl sulfoxide, ethyl acetate, the ether and an acetone] meltable to ****, chloroform, a methylene chloride, benzene, a hexane, and the petroleum ether.
10) Rf value 0.55 is shown in thin-layer chromatography [TLC, ****:precoat silica gel 70F254, 0.2mm, and; expansion solvent:chloroform-ethyl-acetate-ethanol made from WAKO (10:2:0. 007)]. Moreover, Rf value 0.41 is shown in thin-layer chromatography (TLC, ****:antiphase precoat silica gel RP-8F254s,; expansion solvent [by Merck Co.]:95% methanol). If sulfuric-acid solution is sprayed 10% on TLC and it heats, purplish red - brown will be presented.
[0005] Moreover, this invention is formula (II): which is a new ginseng sapogenin. [Formula 2] DAMARA -20 which came out and was named the mulberry JIPANAKISA triol (quasipanaxatriol) expressed, (22), 24-diene - Also offering 3beta, 6alpha, and 12beta-triol (dammara-20 (22) 24-diene-3beta, 6alpha, 12 beta-triol), this sapogenin has the following features.
1) The melting point is 165-168 degrees C.
2) [alpha] It has the optical activity of D20+50 degree (C= 1.0, methanol).
3) It has molecule composition of C30H5003.
4) An infrared absorption spectrum (KBr, cm-1) has 3450, 2960, 1435, 1390, and the absorption maximum peculiar to 1050.
5) A mass analysis spectrum (Fab, m/z) shows 457- (M-H).
7) 13C nuclear-magnetic-resonance spectrum (d5-pyridine) 39.6 (C-1) 28.2 (C-2) and 78.5 (C-3), 40.4 (C-4), 61.9 (C-5), and 67.8 (C-6), 47.8 (C-7), 41.5 (C-8), and 50.6 (C-9), 39.6 (C-10), 28.9 (C-11), and 72.7 (C-12), 50.8 (C-13), 50.9 (C-14), and 32.3 (C-15), 28.2 (C-16), 50.5 (C-17), and

17.7 (C-18), 17.5 (C-19), 140.2 (C-20), and 13.2 (C-21), The signal of 123.6 (C-22), 27.5 (C-23), 123.9 (C-24), 135.3 (C-25), 25.7 (C-26), 17.7 (C-27), 32.0 (C-28), 16.5 (C-29), and 17.2 (C-30) is shown.
8) There is no smell and it is colorless needle crystal (from methanol-water to crystallization).
9) It is [a methanol, ethanol, n-butanol, 2-propanol, n-propanol a pyridine, dimethyl sulfoxide, ethyl acetate, the ether and an acetone] meltable to ****, chloroform, a methylene chloride, benzene, a hexane, and the petroleum ether.
10) Rf value 0.45 is shown in thin-layer chromatography [TLC, ****:precoat silica gel 70F254, 0.2mm, and; expansion solvent:chloroform-ethyl-acetate-ethanol made from WAKO (1:1:0. 015)]. Moreover, Rf value 0.31 is shown in thin-layer chromatography (TLC, ****:antiphase precoat silica gel RP-8F254s, ; expansion solvent [by Merck Co.]:85% methanol). If sulfuric-acid solution is sprayed 10% on TLC and it heats, purplish red - brown will be presented.
[0006] And by hydrolyzing and dehydration processing the glycoside which makes a skeleton 20(S)-protopanaxadiol and 20(S)-protopanaxadiol, this invention offers the manufacturing method of the mulberry JIPANAKISA diol (formula I) obtained as a sapogenin, and mulberry JIPANAKISA triol (formula II), and explains it in detail below. That is, a solvent extracts the terrestrial part, the root, and its tissue culture object of a medicinal ginseng, especially Panax schinseng (PANAKKUSU GINSENGU, SHI A MEIYA) to the method of this invention, the mixture (total GINSENOSHIDO is called below) of the glycoside (GINSENOSHIDO) which makes a skeleton 20(S)-protopanaxadiol and 20(S)-protopanaxadiol is divided into it, and the method of performing hydrolysis and dehydration processing to this, and manufacturing the mulberry JIPANAKISA diol and mulberry JIPANAKISA triol which are the specified substance of this invention is included In the method of this invention, extraction after degreasing processing is first performed using a usual lipophilicity organic solvent like n-hexane, without carrying out degreasing processing of the medicinal ginseng of a raw material.
a) Maceration of the raw material is carried out to the lower alcohol like a methanol and ethanol, if it is with the need, it will heat, and filter, and obtain an extract. You may perform this extract operation repeatedly if needed. These extracts are doubled, a solvent is distilled off under reduced pressure, and it considers as extraction extractives. These extraction extractives are distributed to n-hexane and water, the ether extracts the water layer section further if needed, the water layer section is extracted by n-butanol, a solvent is distilled off

under reduced pressure of the solvent of n-butanol section, and total GINSENOSHIDO is obtained as n-butanol extractives.
b) Maceration of the raw material is carried out to the lower alcohol like a methanol and ethanol, if it is with the need, it will heat, and filter, and obtain an extract. You may perform this extract operation repeatedly if needed. These extracts are doubled, a solvent is distilled off under reduced pressure, and it considers as an extraction extract. This extraction extract is distributed to n-hexane and water, the ether extracts the water layer section further if needed, and the water layer section is contacted to the polystyrene system resin adsorbents (for example, diamond ion HP-20, Mitsubishi Kasei Corp. make, etc.) with which the bridge was constructed over porosity by the huge network structure. These adsorbents are five to 10 time ********** of the amount of content saponins. Subsequently, a resin is often washed with water, it elutes and condenses with a lower alcohol like a methanol or ethanol, or about 30% or more of lower-alcohol content water, and total GINSENOSHIDO is obtained.
[0007] Various kinds of ginseng saponins contain total GINSENOSHIDO obtained by the above methods, and the mulberry JIPANAKISA diol and mulberry JIPANAKISA triol of the quality of the specified substance are manufactured by the following method after this.
a) Dissolve total GINSENOSHIDO in water, add rough hesperidinase or the sucroclastic enzyme like the man intestinal-bacteria origin, and an enzymatic hydrolysis reaction removes one day or the joint sugar which was made to carry out a churning reaction for two days, and it has combined with the glucose machine of the 3rd place and the 20th place of each ginsenoside at about 36-37 degrees C. If sugar is removed, precipitation will generate. subsequently, the thing for which the acetic acid of reaction mixture and the equivalent is added and heated -- or a concentrated hydrochloric acid, a concentrated sulfuric acid, concentrated nitric acid, or the hydroxyl group that added the trifluoroacetic acid and has been combined with the 20th place a room temperature or by heating so that it may become 0.5% is removed by dehydration so that it may become 0.1 conventions
b) total GINSENOSHIDO is dissolved in water, a lower alcohol, or a water lower alcohol, and it becomes 0.1 conventions -- as -- a concentrated hydrochloric acid, a concentrated sulfuric acid, and concentrated nitric acid -- or add a trifluoroacetic acid so that it may become 0.5%, remove the joint sugar combined with the 20th place of each GINSENOSHIDO a room temperature or by heating by the adding-water decomposition reaction, and remove a hydroxyl group by dehydration simultaneously The ether

extracts the obtained reaction mixture, an extract is neutralized in saturation sodium-hydrogencarbonate solution, subsequently, the washing back is distilled off with water and a solvent is distilled off by reduced pressure.
The concentrate obtained by the above-mentioned method of a or b is given to silica gel column chromatography [for example, the silica gel by Merck Co. and 60 - 230 mesh; elution solvent:chloroform-ethyl-acetate-ethanol (1:1:0. 015)], separation refining is carried out and a mulberry JIPANAKISA diol and mulberry JIPANAKISA triol are obtained. These each is recrystallized in the mixed solvent like methanol-water, and a mulberry JIPANAKISA diol and mulberry JIPANAKISA triol are obtained.
[0008] Moreover, the mulberry JIPANAKISA diol and mulberry JIPANAKISA triol of this invention Besides above-mentioned Panax schinseng (PANAKKUSU GINSENGU, SHI A MEIYA) 37 ginsengs (PANAKKUSU PUSOIDOGINSENGU, WARIHHI or Panax notoginseng, par kill) and U.S. ginseng (PANAKKUSU KINKIYUHORIUMU, Linne) which contain GINSENOSHIDO Rb1 and Rd so much, And GINSENOSHIDO Rb2 Genus Panax vegetation, such as Panax japonicus (PANAKKUSU YAPONIKA, SHI A MEIYA) to contain and the Himalaya ginseng (PANAKKUSU PUSOIDOGINSENGU, ZUBUSUBU or the Ricinus communis rye CHIUSU PAL, ANGUSUTEIFURORIIUSU), is used as a raw material. by the aforementioned method It can obtain. moreover -- 20 -- (-- S --) - protopanaxadiol -- a nucleus -- a skeleton -- ** -- carrying out -- having been similar -- sugar -- coordination -- having -- a saponin -- containing -- others -- vegetation -- [-- for example, -- Cucurbitaceae -- flax -- CHAZURU (GINOSUTEMUMA pen TAFIRURUMU Makino and its relative vegetation) --] -- from -- the above -- a method -- it can obtain .
[0009] Furthermore, the mulberry JIPANAKISA diol and mulberry JIPANAKISA triol in this invention are very useful as a reinforcement agent which reinforces the chemotherapy-effect of an anticancer agent remarkably by making the susceptibility over the anticancer agent of the cancer cell which gained drug resistance increase, and canceling drug resistance.
[0010] In this case, the cancer cells which the susceptibility over an anticancer agent increases by the reinforcement agent in this invention are adenocarcinoma cells including a suprarenal gland, the kidney, liver, and a large intestine organization, a pancreatic cancer cell, a kind carcinoma cell, the chronic myelogenous leukemia cell in a plastic strike crisis, a non-smallness cellularity lung cancer cell, a neuroblastoma cell, a pheochromocytoma cell, an adult acute lymphoblastosis cell, a tubercle **** specialization lymphoma cell, a breast cancer cell, and an ovarian cancer cell.

[0011] Moreover, the anticancer agents which an anticancer operation reinforces by the reinforcement agent in this invention are BINKA alkaloid, EPIPODOFIRO toxin, an anthracycline system antibiotic, actinomycin D, PURIKA mycin, a puromycin, Gramicidin D, taxol, Corte Singh, a cytochalasin B, an emetine, MEITANSHIN, and an AMUSA curine. More desirable one is BINKA. They are alkaloid, EPIPODOFIRO toxin, an anthracycline system antibiotic, actinomycin D, and PURIKA mycin.
[0012] Time to differ simultaneously with an anticancer agent is medicated with the reinforcement agent in this invention. In the case of the latter, a reinforcement agent is prescribed for the patient at near time as it is enough to reinforce the effect of a medicine of an anticancer agent.
[0013] the case of internal use of as opposed to [although the amounts of medication of the reinforcement agent in this invention differ according to condition of disease] an adult -- one day -- 1 time or several times -- dividing -- 1-50mg / one day / 60kg weight -- they are 3-15mg / one day / 60kg weight preferably
[0014] The reinforcement agent by this invention consists of an excipient of the active principle simple substance of this invention, an active principle and a solid-state, or a liquid. And as a pharmaceutical form of medication, the form of internal use, such as powder, a tablet, a suspension agent and an emulsion, a capsule, a granule, a troche agent, a round-head agent, liquid medicine, a spirits-of-wine agent, a syrup agent, and a RIMONAZE agent, is usually in the prescribing [for the patient]-a medicine method row. Moreover, in-the-living-body pouring may be carried out in the form of an injection agent, or you may be external application in the form of an ointment, a plaster, liquid medicine, powder, a cypripedium agent, a suppository, an aerosol agent, a PAPPU agent, a SONIMENTO agent, a lotion agent, an enema agent, an emulsion, etc. As an excipient of the solid-state used here or a liquid, a well-known thing is used in the field concerned. However, it is desirable to tablet-ize so that the active principle of this invention required for 1 time of the amount of medication which was mentioned above may be included.
[0015] If some examples are given, as an excipient in powder and the other powdered material for internal use A lactose, a crystalline cellulose, starch, a dextrin, calcium phosphate, a calcium carbonate, Composition and a natural aluminum silicate, a magnesium oxide, a dryness aluminum hydroxide, A magnesium stearate, a sodium bicarbonate, etc. are mentioned. In the case of external application powder, a zinc stearate, a magnesium stearate, a magnesium carbonate, a precipitated

calcium carbonate, the following ****** bismuth, the end of a potassium aluminum sulfate, etc. are mentioned in a zinc oxide, talc, starch, a kaolin, and the end of a boric acid. As an excipient in liquid medicine, water, a glycerol, a propylene glycol, single syrup, ethanol, fatty oil, ethylene glycol, a polyethylene glycol, a sorbitol, etc. are mentioned. Furthermore, in the case of an ointment, the hydrophobic radical agent made combining a fat, fatty oil, lanolin, vaseline, a glycerol, yellow bees wax, Japan wax, paraffin, a liquid paraffin, a resin, higher alcohol, plastics, glycols, water, the surfactant, etc. or a hydrophilic radical agent (an emulsion nature basis, a water-soluble basis, and a suspensibility basis are included) is used as an excipient.

[0016] Although the following examples are offered in order to explain this invention concretely, you should not interpret, if it receives restrictions.

[Example]

After shredding the terrestrial part (the product from South Korea, 6kg) of example 1 Panax schinseng, maceration of the methanol (56L) was added and carried out several weeks. It filtered and the methanol extract was obtained, the methanol (48L) was newly added to the residue, and maceration extraction was carried out. Same operation was performed a total of 3 times, the obtained methanol extract was doubled, solvent distilling off was carried out by reduced pressure, and methanol extraction extractives (1.15kg) were obtained. this methanol extraction extract (0.84kg) -- n-hexane and water (2:1. 2L) -- distributing -- the water layer section -- the ether (1L) -- subsequently it extracted by n-butanol (2L) Solvent distilling off of the n-butanol section was carried out by reduced pressure, and total GINSENOSHIDO (0.39kg) was obtained. This total GINSENOSHIDO (5g) was dissolved in the purified water (50ml), rough hesperidinase (0.5g) was added, and it agitated at 37 degrees C for 24 hours. Furthermore, the acetic acid (50ml) was added to this reaction mixture, and it heated at 70 degrees C for 4 hours. Reaction mixture was diluted with water and the ether (50mlx2) extracted. This ether extract is neutralized in saturation sodium-hydrogencarbonate solution, subsequently, the washing back is distilled off with water and a solvent is distilled off by reduced pressure. The obtained extraction extractives (1.2g) were given to the silica gel column chromatography [silica gel [by Merck Co.], 60-230-mesh, and 100g; elution solvent: chloroform-ethyl-acetate-ethanol (10:2:0. 07)], and the antiphase silica gel column chromatography (silica gel RP- 18, 300g; elution solvent : 85-90% methanol), separation refining was carried out, it crystallized from the water-methanol, and a mulberry JIPANAKISA diol (38mg) and mulberry

JIPANAKISA triol (100mg) were

[0017] Set in the example 2 example 1. The Homo sapiens enterobacterium which dissolved in the peptone-yeast-extract culture medium (g/L, a peptone, 10; yeast extract, 5; L-cysteine hydrochloride monohydrate, a 0.5; sodium chloride, 6.8; potassium chloride, a 0.4; calcium chloride, 0.2; magnesium sulfate, 0.094; phosphoric-acid 1 hydrogen sodium, a 0.06; potassium dihydrogenphosphate, 0.06) (1000 ml), and carried out subculture of obtained total GINSENOSHIDO (10g) by the general culture medium for anaerobic bacteria (for example, GAM culture medium) was added, and the anaerobic culture was carried out at 36 degrees C for 48 Reaction mixture was diluted with water and ethyl acetate (200ml) extracted. The ethyl-acetate section was distilled off with water, the solvent was distilled off by reduced pressure after washing, it dissolved in acetic-acid solution (100ml) 50%, and the obtained extraction extractives (1.9g) were heated at 70 degrees C for 4 hours. Reaction mixture was diluted with water and the ether (100mlx2) extracted. This ether extract is neutralized in saturation sodium-hydrogencarbonate solution, subsequently, the washing back is distilled off with water and a solvent is distilled off by reduced pressure. The obtained extraction extractives were given to the silica gel column chromatography [silica gel [by Merck Co.], 60-230-mesh, and 200g; elution solvent: chloroform-ethyl-acetate-ethanol (10:2:0. 07)], and the antiphase silica gel column chromatography (silica gel RP[by Merck Co.]- 18,300g; elution solvent : 85-90% methanol), separation refining was carried out, it crystallized from the water-methanol, and a mulberry JIPANAKISA diol (1.0g) and mulberry JIPANAKISA triol (2.7g)

[0018] After shredding the root (the product from South Korea, 2.7kg) of example 3 Panax schinseng, ethanol (5L) was added 50% and it flowed back. It filtered and the ethanol extract was obtained, ethanol (5L) was newly added to the residue, and reflux extraction was carried out. Same operation was performed a total of 3 times, the obtained ethanol extract was doubled, solvent distilling off was carried out by reduced pressure, and ethanol extraction extractives (900g) were obtained. These ethanol extraction extractives (300g) were distributed in water (3L), and acetic-acid extractives (0.5Lx2) extracted the water layer section. The polystyrene system resin adsorbent (diamond ion HP-20, Mitsubishi Kasei Corp. make) by which the bridge was constructed after extraction in the water layer section, and was constructed over porosity by the huge network structure in the water layer section was made to contact with ethyl acetate (2L, 1L, 1L), the resin was often washed with water, next, it eluted with the methanol and the methanol 50%, the methanol elution

section was condensed, and total GINSENOSHIDO (35g) was obtained. This total GINSENOSHIDO (10g) was dissolved in decinormal hydrochloric-acid-ethanol (100ml), and it heated at 80 degrees C for 2 hours. The ethanol of reaction mixture was distilled off by reduced pressure, it diluted with water, and the ether (100mlx2) extracted. This ether extract is neutralized in saturation sodium-hydrogencarbonate solution, subsequently, the washing back is distilled off with water and a solvent is distilled off by reduced pressure. The obtained extraction extractives were given to the silica gel column chromatography [silica gel [by Merck Co.], 60-230-mesh, and 200g; elution solvent: chloroform-ethyl-acetate-ethanol (10:2:0. 07)], and the antiphase silica gel column chromatography (silica gel RP[by Merck Co.]- 18, 300g; elution solvent : 85-90% methanol), separation refining was carried out, it crystallized from the water-methanol, and a mulberry JIPANAKISA diol (0. 73g) and mulberry JIPANAKISA triol (0. 29g)

[0019] A lactose, a crystalline cellulose, and 1% of magnesium stearates were added to example of tablet 1 mulberry JIPANAKISA diol, or mulberry JIPANAKISA triol 30mg, and it mixed uniformly, and tableted using the tableting machine, and the tablet with one lock of 200mg was obtained.

[0020] Moisture was removed and the injection was obtained, after filling up the vial which sterilized with the solution of example of tablet 2 mulberry JIPANAKISA diol, or mulberry JIPANAKISA triol 15mg, and the polysorbate 80 in sterile.

[0021] Moisture was removed and the injection was obtained, after filling up the vial which sterilized with example of tablet 3 mulberry JIPANAKISA diol, or mulberry JIPANAKISA triol 15mg, 1mg of vinblastine sulfate, and the solution of the polysorbate 80 in sterile.

[0022] in vitro cytotoxicity a in the leukemic cell stock (P388) of the mouse of an example 4 reinforcement agent The leukemic cell stock (P388) and its adriamycin (ADM) resistant-cell stock (P388/ADM) of a mouse were used for the examination of the examining method **. This resistant-cell stock (P388/ADM) was about 180 time resistance to about 80 times and vinblastine (VBL) to the daunomycin (DAU) as compared with the parent-cell stock (P388). The resistant-cell stock (P388/ADM) (1x106 pieces) was cultivated under 5% carbon dioxide of steam saturation for 48 hours in the culture medium (20microM mercaptoethanol, 10% fetal-calf-serum content RPMI1640 culture medium) containing a reinforcement agent (12. 5-50microM) and the anticancer agent which carried out two fold serial dilution. Moreover, apart from it, the resistance cancer cell of a same number individual was cultivated to the culture medium only containing an anticancer agent, and it considered as the control group (control) at

it. Each number of proliferation cancer cells was measured, and 50% growth inhibition concentration (IC50) of an anticancer agent was computed. And the index of a drug tolerance was computed by the following formula.

Drug-tolerance (index RF) =IC50(P388/ADM)/IC50 (P388)

b) A test-result reinforcement agent mulberry JIPANAKISA diol and mulberry JIPANAKISA triol are the concentration which does not show cytotoxicity, and decreased more remarkably than the verapamil used as a contrast medicine the index of the drug tolerance of a resistant-cell stock (P388/ADM) to an anticancer agent daunomycin (DAU) and vinblastine (VBL), consequently canceled the drug tolerance of a resistant-cell stock.

[Table 1]

表1. クワジバナキサジオール(Qpd)及びクワジバナキサトリオール(Qpt)のP388/ADM細胞の DAU及びVBL感受性に対する影響

増強剤	細胞毒性	濃度	細胞成長率	RF	
	IC50 (μM)	(μM)	(%)	DAU	VBL
コントロール			100	79±3	180±2
ベラパミル	45.5	6.25	95	6.9±0.1	9.7±0.1
Qpd	45.3	25	97	1.2±0.1	2.9±0.2
		37.5	80	0.65±0.01	0.87±0.02
Qpt	77.4	6.25	99	43±0.1	31±0.4
		12.5	98	27±0.2	19±0.7
		25	101	8.8±0.1	11±0.1
		50	94	1.7±0.1	4.9±0.2

The mulberry JIPANAKISA diol and mulberry JIPANAKISA triol of this invention reinforce the effect of a medicine of an anticancer agent, and it is in Ming to cancel a drug tolerance, therefore it can be used for them as a reinforcement agent so that the above result may show.

[0023]

[Effect of the Invention] The mulberry JIPANAKISA diol and mulberry JIPANAKISA triol in this invention are very useful as a reinforcement agent which reinforces the chemotherapy-effect of an anticancer agent remarkably by making the susceptibility over the anticancer agent of the cancer cell which gained drug resistance increase, and canceling drug resistance.

[Translation done.]

TECHNICAL FIELD

[Industrial Application] this invention relates to the reinforcement agent for increasing the susceptibility of the cancer over the anticancer agent which makes an active principle a new ginseng sapogenin, its manufacturing method, and its new ginseng sapogenin.

[Translation done.]

PRIOR ART

[Description of the Prior Art] Since the susceptibility of Kanji by use of the chemotherapic drug of leukemia, a lymphoma, and various cancers like a solid cancer over each anticancer agent of a cancer cell is uneven, he is rare. The chemotherapy of cancer goes wrong also for the endogenicity resistance to the ** agent treatment of cancer. In other cases, there are resistance and a bird clapper to the anticancer agent for which cancer was used in former treatment. The curative effect of these medicines is then removed. A still more important problem may show resistance also to other anticancer agents which are unrelated to the medicine with which the cancer which recurred was before used by either about [that it is resistance] the chemical structure or the action mechanism to the anticancer agent used in former treatment. These phenomena are put together, are called drug resistance, and are participating in clinical cancer treatment widely.

[Translation done.]

EFFECT OF THE INVENTION

[Effect of the Invention] The mulberry JIPANAKISA diol and mulberry JIPANAKISA triol in this invention are very useful as a reinforcement agent which reinforces the chemotherapy-effect of an anticancer agent remarkably by making the susceptibility over the anticancer agent of the cancer cell which gained drug resistance increase, and canceling drug resistance.

[Translation done.]

TECHNICAL PROBLEM

[Problem(s) to be Solved] In the present condition of such cancer chemotherapy, in order to reduce side effects, such as bone marrow suppression which conquers drug resistance and an anticancer agent has, it is pressing need to develop the medicine which reinforces the effectiveness of an anticancer agent. They are BERAPAMIRU [Tsuruo and T. as a thing typical to the medicine used for this purpose.; Cancer Res. 41, 1967 (1981)], and cyclosporin A[Slater, L.; Although there are J. Clin. Invest. 77 and 1405 (1986)], clinical application is made difficult for the side effect. They separated the rough saponin of a medicinal ginseng from the decomposition product obtained hydrolysis and by carrying out dehydration, the artificers of this invention were process search the medicine which reinforces the effect of a medicine of an anticancer agent, when the new ginseng sapogenin of structure non-Ming which named it a mulberry JIPANAKISA diol and mulberry JIPANAKISA triol increases the susceptibility of the cancer over an anticancer agent, they canceled drug resistance, and they found out reinforcing the chemotherapy-effect of an anticancer agent remarkably. Then, the chemical structure of the ginseng sapogenin of the structure non-Ming was determined, and since the method that they could be produced so much industrially was invented, it came to complete this invention with the operation which reinforces the effect of a medicine of those anticancer agents.

[Translation done.]

MEANS

[Means for Solving the Problem] That is, this invention is formula (I): which is a new ginseng sapogenin. [Formula 1] DAMARA -20 (22), 24-diene which came out and were named the mulberry JIPANAKISA diol (quasipanaxadiol) expressed - Offering a 3beta and 12beta-diol (dammara-20 (22) 24-diene-3beta, 12 beta-diol), this sapogenin has the following features.

1) The melting point is 150-153 degrees C.

2) [alpha] D20 It has the optical activity of +15 degree (C= 1.0,

methanol).
3) It has molecule composition of C30H5002.
4) An infrared absorption spectrum (KBr, cm-1) has 3400, 2900, 1450, 1380, and the absorption maximum peculiar to 1020.
5) A mass analysis spectrum (Fab, m/z) shows 441- (M-H).
7) 13C nuclear-magnetic-resonance spectrum (d5-pyridine) 39.3 (C-1), 27.0 (C-2), 78.5 (C-3), 40.4 (C-4), 56.4 (C-5), and 18.5 (C-6), 35.3 (C-7), 37.1 (C-8), and 50.9 (C-9), 39.6 (C-10), 32.2 (C-11), and 71.9 (C-12), 50.4 (C-13), 51.2 (C-14), and 32.6 (C-15), 26.8 (C-16), 51.2 (C-17), and 16.8 (C-18), 16.5 (C-19), 140.2 (C-20), and 27.4 (C-21), The signal of 123.8 (C-22), 30.0 (C-23), 125.4 (C-24), 131.5 (C-25), 25.7 (C-26), 17.7 (C-27), 28.2 (C-28), 15.8 (C-29), and 17.0 (C-30) is shown.
8) There is no smell and it is colorless needle crystal (from methanol-water to crystallization).
9) It is [a methanol, ethanol, n-butanol, 2-propanol, n-propanol a pyridine, dimethyl sulfoxide, ethyl acetate, the ether and an acetone] meltable to ****, chloroform, a methylene chloride, benzene, a hexane, and the petroleum ether.
10) Rf value 0.55 is shown in thin-layer chromatography [TLC, ****:precoat silica gel 70F254, 0.2mm, and; expansion solvent:chloroform-ethyl-acetate-ethanol made from WAKO (10:2:0. 007)]. Moreover, Rf value 0.41 is shown in thin-layer chromatography (TLC, ****:antiphase precoat silica gel RP-8F254s,; expansion solvent [by Merck Co.]:95% methanol). If sulfuric-acid solution is sprayed 10% on TLC and it heats, purplish red - brown will be presented.
[0005] Moreover, this invention is formula (II): which is a new ginseng sapogenin. [Formula 2] DAMARA -20 which came out and was named the mulberry JIPANAKISA triol (quasipanaxatriol) expressed, (22), 24-diene - Also offering 3beta, 6alpha, and 12beta-triol (dammara-20 (22) 24-diene-3beta, 6alpha, 12 beta-triol), this sapogenin has the following features.
1) The melting point is 165-168 degrees C.
2) [alpha] It has the optical activity of D20+50 degree (C= 1.0, methanol).
3) It has molecule composition of C30H5003.
4) An infrared absorption spectrum (KBr, cm-1) has 3450, 2960, 1435, 1390, and the absorption maximum peculiar to 1050.
5) A mass analysis spectrum (Fab, m/z) shows 457- (M-H).
7) 13C nuclear-magnetic-resonance spectrum (d5-pyridine) 39.6 (C-1) 28.2 (C-2) and 78.5 (C-3), 40.4 (C-4), 61.9 (C-5), and 67.8 (C-6), 47.8 (C-7), 41.5 (C-8), and 50.6 (C-9), 39.6 (C-10), 28.9 (C-11), and 72.7 (C-12), 50.8 (C-13), 50.9 (C-14), and 32.3 (C-15), 28.2 (C-16), 50.5 (C-17), and

17.7 (C-18), 17.5 (C-19), 140.2 (C-20), and 13.2 (C-21), The signal of 123.6 (C-22), 27.5 (C-23), 123.9 (C-24), 135.3 (C-25), 25.7 (C-26), 17.7 (C-27), 32.0 (C-28), 16.5 (C-29), and 17.2 (C-30) is shown.
8) There is no smell and it is colorless needle crystal (from methanol-water to crystallization).
9) It is [a methanol, ethanol, n-butanol, 2-propanol, n-propanol a pyridine, dimethyl sulfoxide, ethyl acetate, the ether and an acetone] meltable to ****, chloroform, a methylene chloride, benzene, a hexane, and the petroleum ether.
10) Rf value 0.45 is shown in thin-layer chromatography [TLC, ****:precoat silica gel 70F254, 0.2mm, and; expansion solvent:chloroform-ethyl-acetate-ethanol made from WAKO (1:1:0. 015)]. Moreover, Rf value 0.31 is shown in thin-layer chromatography (TLC, ****:antiphase precoat silica gel RP-8F254s,; expansion solvent [by Merck Co.]:85% methanol). If sulfuric-acid solution is sprayed 10% on TLC and it heats, purplish red - brown will be presented.
[0006] And by hydrolyzing and dehydration processing the glycoside which makes a skeleton 20(S)-protopanaxadiol and 20(S)-protopanaxadiol, this invention offers the manufacturing method of the mulberry JIPANAKISA diol (formula I) obtained as a sapogenin, and mulberry JIPANAKISA triol (formula II), and explains it in detail below. That is, a solvent extracts the terrestrial part, the root, and its tissue culture object of a medicinal ginseng, especially Panax schinseng (PANAKKUSU GINSENGU, SHI A MEIYA) to the method of this invention, the mixture (total GINSENOSHIDO is called below) of the glycoside (GINSENOSHIDO) which makes a frame 20(S)-protopanaxadiol and 20(S)-protopanaxadiol is divided into it, and the method of performing hydrolysis and dehydration processing to this, and manufacturing the mulberry JIPANAKISA diol and mulberry JIPANAKISA triol which are the specified substance of this invention is included In the method of this invention, extraction after degreasing processing is first performed using a usual lipophilicity organic solvent like n-hexane, without carrying out degreasing processing of the medicinal ginseng of a raw material.
a) Maceration of the raw material is carried out to the lower alcohol like a methanol and ethanol, if it is with the need, it will heat, and filter, and obtain an extract. You may perform this extract operation repeatedly if needed. These extracts are doubled, a solvent is distilled off under reduced pressure, and it considers as an extraction extract. This extraction extract is distributed to n-hexane and water, the ether extracts the water layer section further if needed, the water layer section is extracted by n-butanol, a solvent is distilled off under

reduced pressure of the solvent of n-butanol section, and total GINSENOSHIDO is obtained as an n-butanol extract.
b) Maceration of the raw material is carried out to the lower alcohol like a methanol and ethanol, if it is with the need, it will heat, and filter, and obtain an extract. You may perform this extract operation repeatedly if needed. These extracts are doubled, a solvent is distilled off under reduced pressure, and it considers as an extraction extract. This extraction extract is distributed to n-hexane and water, the ether extracts the water layer section further if needed, and the water layer section is contacted to the polystyrene system resin adsorbents (for example, diamond ion HP-20, Mitsubishi Kasei Corp. make, etc.) with which the bridge was constructed over porosity by the huge network structure. These adsorbents are five to 10 time ********** of the amount of content saponins. Subsequently, a resin is often washed with water, it elutes and condenses with a lower alcohol like a methanol or ethanol, or about 30% or more of lower-alcohol content water, and total GINSENOSHIDO is obtained.
[0007] Various kinds of ginseng saponins contain total GINSENOSHIDO obtained by the above methods, and the mulberry JIPANAKISA diol and mulberry JIPANAKISA triol of the quality of the specified substance are manufactured by the following method after this.
a) Dissolve total GINSENOSHIDO in water, add rough hesperidinase or the sucroclastic enzyme like the man intestinal-bacteria origin, and an enzymatic hydrolysis reaction removes one day or the joint sugar which was made to carry out a churning reaction for two days, and it has combined with the glucose machine of the 3rd place and the 20th place of each ginsenoside at about 36-37 degrees C. If sugar is removed, precipitation will generate. subsequently, the thing for which the acetic acid of reaction mixture and the equivalent is added and heated -- or a concentrated hydrochloric acid, a concentrated sulfuric acid, concentrated nitric acid, or the hydroxyl group that added the trifluoroacetic acid and has been combined with the 20th place a room temperature or by heating so that it may become 0.5% is removed by dehydration so that it may become 0.1 conventions
b) total GINSENOSHIDO is dissolved in water, a lower alcohol, or a water lower alcohol, and it becomes 0.1 conventions -- as -- a concentrated hydrochloric acid, a concentrated sulfuric acid, and concentrated nitric acid -- or add a trifluoroacetic acid so that it may become 0.5%, remove the joint sugar combined with the 20th place of each GINSENOSHIDO a room temperature or by heating by the adding-water decomposition reaction, and remove a hydroxyl group by dehydration simultaneously The ether

extracts the obtained reaction mixture, an extract is neutralized in saturation sodium-hydrogencarbonate solution, subsequently, the washing back is distilled off with water and a solvent is distilled off by reduced pressure.
The concentrate obtained by the above-mentioned method of a or b is given to silica gel column chromatography [for example, the silica gel by Merck Co. and 60 - 230 mesh; elution solvent:chloroform-ethyl-acetate-ethanol (1:1:0. 015)], separation refining is carried out and a mulberry JIPANAKISA diol and mulberry JIPANAKISA triol are obtained. These each is recrystallized in the mixed solvent like methanol-water, and a mulberry JIPANAKISA diol and mulberry JIPANAKISA triol are obtained.
[0008] Moreover, the mulberry JIPANAKISA diol and mulberry JIPANAKISA triol of this invention Besides above-mentioned Panax schinseng (PANAKKUSU GINSENGU, SHI A MEIYA) 37 ginsengs (PANAKKUSU PUSOIDOGINSENGU, WARIHHI or Panax notoginseng, par kill) and U.S. ginseng (PANAKKUSU KINKIYUHORIUMU, Linne) which contain GINSENOSHIDO Rbl and Rd so much, And GINSENOSHIDO Rb2 Genus Panax vegetation, such as Panax japonicus (PANAKKUSU YAPONIKA, SHI A MEIYA) to contain and the Himalaya ginseng (PANAKKUSU PUSOIDOGINSENGU, ZUBUSUBU or the Ricinus communis rye CHIUSU PAL, ANGUSUTEIFURORIIUSU), is used as a raw material. by the aforementioned method It can obtain. moreover — 20 -- (-- S --) - protopanaxadiol -- a nucleus -- a frame -- ** -- carrying out -- having been similar -- sugar — coordination -- having -- a saponin -- containing -- others -- vegetation -- [-- for example, — Cucurbitaceae -- an amateur -- CHAZURU (GINOSUTEMUMA pen TAFIRURUMU Makino and its relative vegetation) --] -- from -- the above -- a method -- it can obtain .
[0009] Furthermore, the mulberry JIPANAKISA diol and mulberry JIPANAKISA triol in this invention are very useful as a reinforcement agent which reinforces the chemotherapy-effect of an anticancer agent remarkably by making the susceptibility over the anticancer agent of the cancer cell which gained drug resistance increase, and canceling drug resistance.
[0010] In this case, the cancer cells which the susceptibility over an anticancer agent increases by the reinforcement agent in this invention are adenocarcinoma cells including a suprarenal gland, the kidney, liver, and a large intestine organization, a pancreatic cancer cell, a kind carcinoma cell, the chronic myelogenous leukemia cell in a plastic strike crisis, a non-smallness cellularity lung cancer cell, a neuroblastoma cell, a pheochromocytoma cell, an adult acute lymphoblastosis cell, a tubercle **** specialization lymphoma cell, a

breast cancer cell, and an ovarian cancer cell.
[0011] Moreover, the anticancer agents which an anticancer operation reinforces by the reinforcement agent in this invention are BINKA alkaloid, EPIPODOFIRO toxin, an anthracycline system antibiotic, actinomycin D, PURIKA mycin, a puromycin, Gramicidin D, taxol, Corte Singh, a cytochalasin B, an emetine, MEITANSHIN, and an AMUSA curine. More desirable one is BINKA. They are alkaloid, EPIPODOFIRO toxin, an anthracycline system antibiotic, actinomycin D, and PURIKA mycin.
[0012] Time to differ simultaneously with an anticancer agent is medicated with the reinforcement agent in this invention. In the case of the latter, a reinforcement agent is prescribed for the patient at near time as it is enough to reinforce the effect of a medicine of an anticancer agent.
[0013] the case of internal use of as opposed to [although the amounts of medication of the reinforcement agent in this invention differ according to condition of disease] an adult -- one day -- 1 time or several times -- dividing -- 1-50mg / one day / 60kg weight -- they are 3-15mg / one day / 60kg weight preferably
[0014] The reinforcement agent by this invention consists of an excipient of the active principle simple substance of this invention, an active principle and a solid-state, or a liquid. And as a pharmaceutical form of medication, the form of internal use, such as powder, a tablet, a suspension agent and an emulsion, a capsule, a granule, a troche agent, a round-head agent, liquid medicine, a spirits-of-wine agent, a syrup agent, and a RIMONAZE agent, is usually in the prescribing [for the patient]-a medicine method row. Moreover, in-the-living-body pouring may be carried out in the form of an injection agent, or you may be external application in the form of an ointment, a plaster, liquid medicine, powder, a cypripedium agent, a suppository, an aerosol agent, a PAPPU agent, a SONIMENTO agent, a lotion agent, an enema agent, an emulsion, etc. As an excipient of the solid-state used here or a liquid, a well-known thing is used in the field concerned. However, it is desirable to tablet-ize so that the active principle of this invention required for 1 time of the amount of medication which was mentioned above may be included.
[0015] If some examples are given, as an excipient in powder and the other powdered material for internal use A lactose, a crystalline cellulose, starch, a dextrin, calcium phosphate, a calcium carbonate, Composition and a natural aluminum silicate, a magnesium oxide, a dryness aluminum hydroxide, A magnesium stearate, a sodium bicarbonate, etc. are mentioned. In the case of external application powder, a zinc

stearate, a magnesium stearate, a magnesium carbonate, a precipitated calcium carbonate, the following ****** bismuth, the end of a potassium aluminum sulfate, etc. are mentioned in a zinc oxide, talc, starch, a kaolin, and the end of a boric acid. As an excipient in liquid medicine, water, a glycerol, a propylene glycol, single syrup, ethanol, fatty oil, ethylene glycol, a polyethylene glycol, a sorbitol, etc. are mentioned. Furthermore, in the case of an ointment, the hydrophobic radical agent made combining a fat, fatty oil, lanolin, vaseline, a glycerol, yellow bees wax, Japan wax, paraffin, a liquid paraffin, a resin, higher alcohol, plastics, glycols, water, the surfactant, etc. or a hydrophilic radical agent (an emulsion nature basis, a water-soluble basis, and a suspensibility basis are included) is used as an excipient.
[0016] Although the following examples are offered in order to explain this invention concretely, you should not interpret, if it receives restrictions.

[Translation done.]

EXAMPLE

[Example]
After shredding the terrestrial part (the product from South Korea, 6kg) of example 1 Panax schinseng, maceration of the methanol (56L) was added and carried out several weeks. It filtered and the methanol extract was obtained, the methanol (48L) was newly added to the residue, and maceration extraction was carried out. Same operation was performed a total of 3 times, the obtained methanol extract was doubled, solvent distilling off was carried out by reduced pressure, and the methanol extraction extract (1.15kg) was obtained. this methanol extraction extract (0.84kg) -- n-hexane and water (2:1. 2L) -- distributing -- the water layer section -- the ether (1L) -- subsequently it extracted by n-butanol (2L) Solvent distilling off of the n-butanol section was carried out by reduced pressure, and total GINSENOSHIDO (0.39kg) was obtained. This total GINSENOSHIDO (5g) was dissolved in the purified water (50ml), rough hesperidinase (0.5g) was added, and it agitated at 37 degrees C for 24 hours. Furthermore, the acetic acid (50ml) was added to this reaction mixture, and it heated at 70 degrees C for 4 hours. Reaction mixture was diluted with water and the ether (50mlx2) extracted. This ether extract is neutralized in saturation sodium-hydrogencarbonate

solution, subsequently, the washing back is distilled off with water and a solvent is distilled off by reduced pressure. The obtained extraction extract (1.2g) was given to silica gel column chromatography [silica gel [by Merck Co.], 60-230-mesh, and 100g; elution solvent: chloroform-ethyl-acetate-ethanol (10:2:0. 07)], and antiphase silica gel column chromatography (silica gel RP- 18, 300g; elution solvent : 85-90% methanol), separation refining was carried out, it crystallized from the water-methanol, and a mulberry JIPANAKISA diol (38mg) and mulberry JIPANAKISA triol (100mg) were obtained.

[0017] Set in the example 2 example 1. The man intestinal bacteria which dissolved in the peptone-yeast-extract culture medium (g/L, a peptone, 10; yeast extract, 5; L-cysteine hydrochloride monohydrate, a 0.5; sodium chloride, 6.8; potassium chloride, a 0.4; calcium chloride, 0.2; magnesium sulfate, 0.094; phosphoric-acid 1 hydrogen sodium, a 0.06; potassium dihydrogenphosphate, 0.06) (1000 ml), and carried out subculture of obtained total GINSENOSHIDO (10g) by the general culture medium for anaerobic bacteria (for example, GAM culture medium) were added, and the anaerobic culture was carried out at 36 degrees C for 48 Reaction mixture was diluted with water and ethyl acetate (200ml) extracted. The ethyl-acetate section was distilled off with water, the solvent was distilled off by reduced pressure after washing, it dissolved in acetic-acid solution (100ml) 50%, and the obtained extraction extract (1.9g) was heated at 70 degrees C for 4 hours. Reaction mixture was diluted with water and the ether (100mlx2) extracted. This ether extract is neutralized in saturation sodium-hydrogencarbonate solution, subsequently, the washing back is distilled off with water and a solvent is distilled off by reduced pressure. The obtained extraction extract was given to silica gel column chromatography [silica gel [by Merck Co.], 60-230-mesh, and 200g; elution solvent: chloroform-ethyl-acetate-ethanol (10:2:0. 07)], and antiphase silica gel column chromatography (silica gel RP[by Merck Co.]- 18, 300g; elution solvent : 85-90% methanol), separation refining was carried out, it crystallized from the water-methanol, and a mulberry JIPANAKISA diol (1.0g) and mulberry JIPANAKISA triol (2.7g) were obtained

[0018] After shredding the root (the product from South Korea, 2.7kg) of example 3 Panax schinseng, ethanol (5L) was added 50% and it flowed back. It filtered and the ethanol extract was obtained, ethanol (5L) was newly added to the residue, and reflux extraction was carried out. Same operation was performed a total of 3 times, the obtained ethanol extract was doubled, solvent distilling off was carried out by reduced pressure,

and the ethanol extraction extract (900g) was obtained. This ethanol extraction extract (300g) was distributed in water (3L), and the acetic-acid extract (0.5Lx2) extracted the water layer section. The polystyrene system resin adsorbent (diamond ion HP-20, Mitsubishi Kasei Corp. make) by which the bridge was constructed after extraction in the water layer section, and was constructed over porosity by the huge network structure in the water layer section was made to contact with ethyl acetate (2L, 1L, 1L), the resin was often washed with water, next, it eluted with the methanol and the methanol 50%, the methanol elution section was condensed, and total GINSENOSHIDO (35g) was obtained. This total GINSENOSHIDO (10g) was dissolved in 0.1 convention hydrochloric-acid-ethanol (100ml), and it heated at 80 degrees C for 2 hours. The ethanol of reaction mixture was distilled off by reduced pressure, it diluted with water, and the ether (100mlx2) extracted. This ether extract is neutralized in saturation sodium-hydrogencarbonate solution, subsequently, the washing back is distilled off with water and a solvent is distilled off by reduced pressure. The obtained extraction extract was given to silica gel column chromatography [silica gel [by Merck Co.], 60-230-mesh, and 200g; elution solvent: chloroform-ethyl-acetate-ethanol (10:2:0. 07)], and antiphase silica gel column chromatography (silica gel RP[by Merck Co.]- 18,300g; elution solvent : 85-90% methanol), separation refining was carried out, it crystallized from the water-methanol, and a mulberry JIPANAKISA diol (0.73g) and mulberry JIPANAKISA triol (0.29g) were obtained

[0019] A lactose, a crystalline cellulose, and 1% of magnesium stearates were added to example of tablet 1 mulberry JIPANAKISA diol, or mulberry JIPANAKISA triol 30mg, and it mixed uniformly, and tableted using the tableting machine, and the tablet with one lock of 200mg was obtained.

[0020] Moisture was removed and the injection agent was obtained, after filling up Bayh Al who sterilized with the solution of example of tablet 2 mulberry JIPANAKISA diol, or mulberry JIPANAKISA triol 15mg, and a polysorbate 80 in sterile.

[0021] Moisture was removed and the injection agent was obtained, after filling up Bayh Al who sterilized with example of tablet 3 mulberry JIPANAKISA diol, or mulberry JIPANAKISA triol 15mg, sulfuric-acid vinblastine 1mg, and the solution of a polysorbate 80 in sterile.

[0022] in vitro cytotoxicity a in the leukemic cell stock (P388) of the mouse of an example 4 reinforcement agent The leukemic cell stock (P388) and its adriamycin (ADM) resistant-cell stock (P388/ADM) of a mouse were used for the examination of the examining method **. This resistant-cell stock (P388/ADM) was about 180 time resistance to about 80 times and

vinblastine (VBL) to the daunomycin (DAU) as compared with the parent-cell stock (P388). The resistant-cell stock (P388/ADM) (1x106 pieces) was cultivated under 5% carbon dioxide of steam saturation for 48 hours in the culture medium (20microM mercaptoethanol, 10% fetal-calf-serum content RPMI1640 culture medium) containing a reinforcement agent (12.5-50microM) and the anticancer agent which carried out two fold serial dilution. Moreover, apart from it, the resistance cancer cell of a same number individual was cultivated to the culture medium only containing an anticancer agent, and it considered as the control group (control) at it. Each number of multiplication cancer cells was measured, and 50% growth inhibition concentration (IC50) of an anticancer agent was computed. And the index of a drug tolerance was computed by the following formula.

Drug-tolerance (index RF) =IC50(P388/ADM)/IC50 (P388)

b) A test-result reinforcement agent mulberry JIPANAKISA diol and mulberry JIPANAKISA triol are the concentration which does not show cytotoxicity, and decreased more remarkably than BERAPAMIRU used as a contrast medicine the index of the drug tolerance of a resistant-cell stock (P388/ADM) to an anticancer agent daunomycin (DAU) and vinblastine (VBL), consequently canceled the drug tolerance of a resistant-cell stock.

[Table 1]

表1. クワジバナキサジオール(Qpd)及びクワジバナキサトリオール(Qpt)のP388/ADM細胞の DAU及びVBL感受性に対する影響

増強剤	細胞毒性	濃度	細胞成長率	RF	
	IC50 (μM)	(μM)	(%)	DAU	VBL
コントロール			100	79±3	180±2
ベラパミル	45.5	6.25	95	6.9±0.1	9.7±0.1
Qpd	45.3	25	97	1.2±0.1	2.9±0.2
		37.5	80	0.65±0.01	0.87±0.02
Qpt	77.4	6.25	99	43±0.1	31±0.4
		12.5	98	27±0.2	19±0.7
		25	101	8.8±0.1	11±0.1
		50	94	1.7±0.1	4.9±0.2

The mulberry JIPANAKISA diol and mulberry JIPANAKISA triol of this invention reinforce the effect of a medicine of an anticancer agent, and it is in Ming to cancel a drug tolerance, therefore it can be used for them as a reinforcement agent so that the above result may show.

[0023]

[Translation done.]